The amyloid beta peptide and alpha synuclein are the main proteins associated with Alzheimer's and Parkinson's disease respectively. In both diseases, interactions between the relevant protein and lipid membranes are hypothesized to be important in disease onset and propagation. We have in earlier studies developed methodology to study kinetics of amyloid fibril formation with high reproducibility [1] and showed that membrane composition affects the kinetics of amyloid beta peptide fibril formation [2]. We now focus on the protein-membrane interaction and study adsorption of alpha synuclein and the amyloid beta peptide to supported lipid bilayers using techniques such as quartz crystal micro balance (QCM), surface plasmon resonance (SPR) and neutron reflectometry. We see a large dependence on electrostatics and to be able to characterize the contributing effects we vary pH and salt concentration in the solution to alter the protein net charge and the electrostatic screening. The lipid bilayer is composed of zwitterionic POPC with or without incorporation of anionic DOPS or Cardiolipin, which in a biologically relevant way present molecular net negative charge of one and two respectively.

1. E. Hellstrand, B. Boland, D. M. Walsh and S. Linse, Ab aggregation produces highly reproducible kinetic data and occurs by a two-phase process, ACS Chem. Neurosci., 2010, 1, 13-18.
2. E. Hellstrand, E. Sparr and S. Linse, Retardation of Abeta Fibril formation by phospholipid vesicles depends on membrane phase behavior, Biophys. J., 2010, 98, 2206-2214.

2531-Pos Board B301

PR3 Interacts Directly to Lipid Bilayers: Evidence from MD Simulations and SPR Experiments

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Proteinase 3 (PR3) is a serine protease of the neutrophils involved in inflammation processes. Its membrane expression is a risk factor for chronic inflammatory diseases. Experimental data led to divergent hypotheses on the binding mode of PR3 to the plasma membrane of neutrophils. While several studies performed on cell lines have identified a number of partner proteins, no direct interaction between hPR3 and any of these potential partners has been demonstrated (reviewed in Hajjar et al, FEBS J., 2010). Differential scanning calorimetry and spectrophotometric measurements, on the other hand, show a direct interaction of PR3 with DMPC vesicles (Goldman et al., Eur J Biochem, 1999).

Using molecular dynamics simulations, we have characterized the membrane-binding site of PR3. Electrostatic surface potential calculations and simulations with an implicit membrane model (IMM1) have showed that PR3 possesses basic amino acids that provide the driving force to orient the protein at the membrane surface, so that a hydrophobic patch can anchor into the hydrophobic region of the membrane. In vitro mutagenesis experiments have confirmed the role of both types of amino acids. All-atom MD simulations and MM/PBSA energy decomposition identify three types of interactions contributing to the anchoring of PR3: hydrogen bonding and charge-pairing with lipid phosphate groups (R177, R186A, K187 and R222), hydrophobic anchoring into the lipid bilayer core (F165, F166 and L223) and cation-π interactions with the choline groups of DMPC (W218).

Surface Plasmon Resonance experiments show a strong binding of Pr3 to DMPC and POPC bilayers.

Altogether our results demonstrate that Pr3 has the ability to directly bind to lipid liposomes and most probably also to the plasma membrane of neutrophils.

2532-Pos Board B302

The Role of Protein Context in Disease-Related Huntingtin Protein/Lipid Interface Interactions

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Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of a poly-glutamine (poly-Q) region near the N-terminus of the huntingtin (htt) protein. Expansion of the poly-Q region above 35-40 repeats results in the disease that is characterized by inclusion body aggregates of mutated protein. The poly-Q region of htt is flanked by a 17 amino acid N-terminal sequence (N17) and a region of proline repeats (P11). As the cell membrane has been proposed to play a role in mediating htt aggregation, Langmuir trough techniques were used to investigate the effects of flanking regions on the surface activity and insertion of htt peptide constructs into a lipid monolayer. Four peptide constructs were tested: N17-Q35-KK, N17-Q35-P11-KK, KK-Q35-KK and KK-Q35-P11-KK, where the additional lysine residues were attached to improve solubility. Surface activity was measured to determine the affinity of each peptide for the air-water interface. The constructs containing the N-terminal sequence had higher surface activities compared to those without. Inclusion of the polyproline region with the N-terminal sequence contributed to the highest surface activity. To determine peptide association with a cell membrane, insertion of each peptide into a monolayer of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) held at a constant surface pressure was also investigated. Peptides with the flanking N-terminus showed appreciable monolayer insertion. The polyproline region only mediated and increased the degree of insertion when the N-terminus was also present. Rates of insertion of the constructs containing the N-terminus were comparable, while those without this region were markedly slower. Experiments were repeated using monolayers of total brain extract to better model a physiological environment with similar results. The amino acid environment surrounding the poly-Q region dramatically affects peptide association with the cell membrane and this may potentially mediate the aggregation process.

2533-Pos Board B303

The Uni- to Multilamellar Transition of Mixed Anionic and Zwitterionic Vesicles Induced by Cytochrome-C: A Small Angle X-Ray Scattering Study

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Lipid-protein interactions are regarded as one of the key factors in several biophysical relevant processes. In the present work, we studied the influence of Cytochrome-c (Cyto-c) on unilamellar vesicles composed by mixing an anionic lipid (the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, POPG, or the cardiolipin, CL) with the zwitterionic lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), by means of small-angle X-ray scattering. Cyto-c is a component of the electron transport chain, where it has a pivotal role in transferring electrons in the inner mitochondrial membrane (IMM), which is composed of a significant amount of anionic lipids. CL is a structurally unique anionic phospholipid, containing two phosphatidylglycerol groups and four acyl chains and it is found predominantly in the IMM. The interaction of Cyto-c and CL-containing mixed vesicles has been extensively studied, and it is appointed as the major responsible for the role of Cyto-c in the cell respiration and also in the cell programmed death (apoptosis), as recently evidenced. Present results show that, in the absence of anionic lipids, Cyto-c is not able to change the structural features of POPC unilamellar vesicles. In the mixed systems, however, Cyto-c induces the formation of a multilamellar vesicles, with a bilayer-bilayer repetition distance of 11 nm, circa 4 nm larger than the staking of POPC multilamellar vesicles. This information indicates that Cyto-c, a globular protein with 3.0 nm of diameter, is probably located among the bilayers. Furthermore, such effect takes place as soon as Cyto-c is mixed in the system, as confirmed by absorption spectroscopy. The time-evolution of such process was also investigated and, interestingly, it is faster in POPG-containing vesicles than in the CL ones. Moreover, the number of staked bilayers is larger in the POPG systems.

2534-Pos Board B304

Molecular Dynamics Prediction and Refinement of Transmembrane Helix Dimers

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Membrane proteins represent around one fourth of all proteins encoded in genomes. Amongst these transmembrane (TM) proteins span the entire lipid bilayer, and -by allowing information to be transmitted across the membrane- play important roles in cell biology and physiology. Several mechanisms for such signal transduction have been revealed, ranging from conformational changes in protein channels and pumps to alterations in helix structures that regulate the activity of intra- and extracellular domains in TM receptors. We have begun to investigate the role of TM helix association in single pass receptors, such as the Eph and plexin receptor. Often the structures of the TM helix associated states are not known and a number of laboratories have been trying to fill this void by use of molecular modeling and dynamics for structure prediction.

This work explores a plausible route to predicting structures of transmembrane helix dimers that combines two layers of modeling. The method is applied to known helix dimer structures as a validation study. Starting from a pair of unfolded peptides, thorough sampling is performed using an implicit bilayer potential to generate starting configurations that are then relaxed in an explicit bilayer. Helix crossing angles for EphA1 and ErbB1/2 dimers obtained from

the explicit bilayer ensembles are in very good agreement with experiment. (Crossing angles from the implicit simulations are incorrect.) The predicted helix packing residues differ from experiment. It is possible the 100 ns simulation times are insufficient for a sampling of helix rotation and thereby a satisfactory determination of the residues involved in helix-helix packing.

2535-Pos Board B305

Highly Pegylated Sterically Stabilized Micelles in Aqueous Media: Structure, Dynamics, and Storage of Therapeutic Agents

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Molecular assemblies of highly PEG-ylated monomers are important in many biomedical applications. For example, sterically stabilized micelles and liposomes of self-assembled DSPE-PEG_{2000} monomers and other phospholipids can serve as biocompatible and relatively nontoxic drug delivery nanocarriers. We perform a detailed study of the micelles formed from DSPE-PEG_{2000} in pure water and isotonic HEPES buffered saline solution [1]. The observed micelle sizes (5 - 15 nm) strongly depend on the solvents and the lipid concentrations used. The critical micelle concentration of DSPE-PEG_{2000} is ~ 10 times higher in water than in buffer and the viscosity of the dispersion dramatically increases with the lipid concentration. To explain the experimentally observed results, we perform atomistic molecular dynamics simulations of the solvated micelles. Our modeling reveals that the observed assemblies have very different aggregation numbers of N ~ 90 in saline solution and N < 8 in water, due to very different screening of the charged phosphate groups in the DSPE-PEG_{2000} monomers. We found that in saline solution the micelle cores can inflate and their PEG coronas highly fluctuate, thus allowing storage and delivery of molecules with different chemistry. We also model the stabilization of model drug molecules and small therapeutic peptides in different regions of the micelle.

[1] Vukovic, L.; Drake, S. D.; Khatib, F. A.; Madriaga, A.; Brandenburg, K. S.; Král, P.; Onyuksel, H. *J. Am. Chem. Soc.* **2011**, *133,* 13481-13488.

2536-Pos Board B306

Effect of Lipid Unsaturation on Membrane Protein Structure and Function from Multi-Microsecond Molecular Dynamics Simulations

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Lipid membrane composition is an important factor in controlling the structure and activity of many membrane proteins. This regulation can take place through the alteration of membrane physico-chemical properties or through direct protein-lipid interactions. Atomistic molecular dynamics simulations on the μs timescale can help uncover the general and specific mechanisms of protein function modulation. omega-3 lipids play key roles in controlling ion channel activity in the brain and heart, with deficiencies associated with a number of a number of health issues, including cardiac and Alzeimer's disease, cognitive function and vision disorders. We have explored the effects of lipid tail unsaturation by carrying out ~10 μs simulations of well-characterized membrane proteins rhodopsin and ion channel KcsA, incorporated into lipid bilayers containing the sn-2 chain with 0 (palmitic), 1 (oleic) and 6 (docosahexaenoic, DHA) double bonds. We observed a marked preference for DHA to solvate the trans-membrane helices of the protein and have identified protein residues preferentially interacting with the unsaturated chains. We will report calculations that reveal the effects of lipid unsaturation on the protein structure and fluctuations, with implications for protein activity. Finally we will discuss ongoing simulations of the KcsA channel in both its closed and open (active) states, to directly uncover the role of lipid unsaturation in function.

2537-Pos Board B307

Water Between Lipids: Domains For Peptides Insertion?

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Capacitance values of monolayers at different areas denote that in a state at which the area per lipid is above a critical value water path are formed beyond the hydration shell of the lipids.
This area increase is 12% of the area for lipids in the expanded state and is comparable to that at which the insertion of proteins and peptides takes place in a variety of lipid composition. Therefore, it is concluded that water paths are formed by expansion implying the entrance of few water molecules into the lipid network. Interestingly some aminoacids having biological activity are able to induce those water paths by interacting with specific groups of the lipids, such as the amine groups in ethanolamines. The kinetics of formation; the thermodynamic and structural properties of those water pockets in the restricted microenvironments framed by lipid groups and its relevance in the selective modulation of the protein-membrane interaction is discussed considering the amount and the state of water induced by the different kinds of groups at the interface region that may act as donor or acceptors in H-bonds, for instance, PO, CO and NH. The analysis is made considering surface pressure and capacitance changes in monolayers at different areas and compared with structural data obtained by means of standard FTIR and biodimensional infrared spectroscopy.
We will use this information for a further insight on the insertion of positively charged peptides into lipid membranes as described by molecular dynamics.

Membrane Fusion

2538-Pos Board B308

SNARE-Mediated Fusion Pore Dynamics from Quantitative TIRF Microscopy

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SNARE proteins are involved in most intracellular fusion events. SNAREs drive complete fusion but are also thought to generate dynamic flickering pores and reversible fusion. Live cell amperometry and simultaneous capacitance recordings indicated that the characteristic pre-spike foot may correspond to a flickering pore which later opens completely (Alvarez de Toledo et al., Nature, 1993). Here we study fusion and pore statistics between SNARE-reconstituted vesicles and supported bilayers (SBLs) using quantitative total internal reflection fluorescence microscopy (TIRFM) with single-lipid resolution. Based on the intensities of the fluorescent lipid-labeled vesicles and the course of the time-dependent intensity increase upon fusion as lipids diffuse through the pore into the SBL, we determined vesicle sizes, the delay time to create the initial pore, and the rate of intermembrane lipid mixing through the pore. These measurements required us to develop a quantitative image analysis algorithm which accounts for TIRFM effects including the spatial decay of incident light, polarization effects, fluorescence dequenching and bleaching. In cholesterol-free vesicles, lipid transfer from the vesicle to the SBL was slower than would be expected were the fusion pore fully open, suggesting that the pore flickers between open and closed states. To quantify these effects we developed a mathematical model of the stochastic fusion pore and the passage of lipids from vesicle to SBL through the pore. Combining the model and experimental measurements, we infer the fraction of time for which the pore is open. Without cholesterol, the pore favors the closed state. We find cholesterol has profound effects: it decreases the delay time between docking and fusion and, once formed, the pore remains fully open. Thus, our results suggest that cholesterol favors the open pore state. Additionally, we report measurements of vesicle curvature-dependence of fusion and pore flickering.

2539-Pos Board B309

Cooperativity of SNARE Complexes in Membrane Fusion: Mechanisms of SNARE Cluster-Mediated Docking and Fusion

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SNARE proteins are the core of the cellular fusion machinery. Vesicle v-SNAREs engage target membrane t-SNAREs and form SNARE complexes thought to pull the membrane surfaces together and drive fusion. Evidence suggests that many SNARE complexes participate in single fusion events and in SNARE-reconstituted vesicle-supported bilayer fusion assays 5-11 complexes were required for fusion [Karatekin et al, PNAS, 2010]. However a mechanistic understanding of this requirement is lacking. We have developed a mathematical model of SNARE cluster-driven docking and fusion which explicitly accounts for interactions between the participating SNARE complexes and the intermembrane forces that must be overcome to trigger fusion. The model is analyzed by a combination of analytical methods and computer simulations. We find that the energetically-favored cluster configuration is a ring of completely assembled SNARE complexes which dock the vesicle to the target